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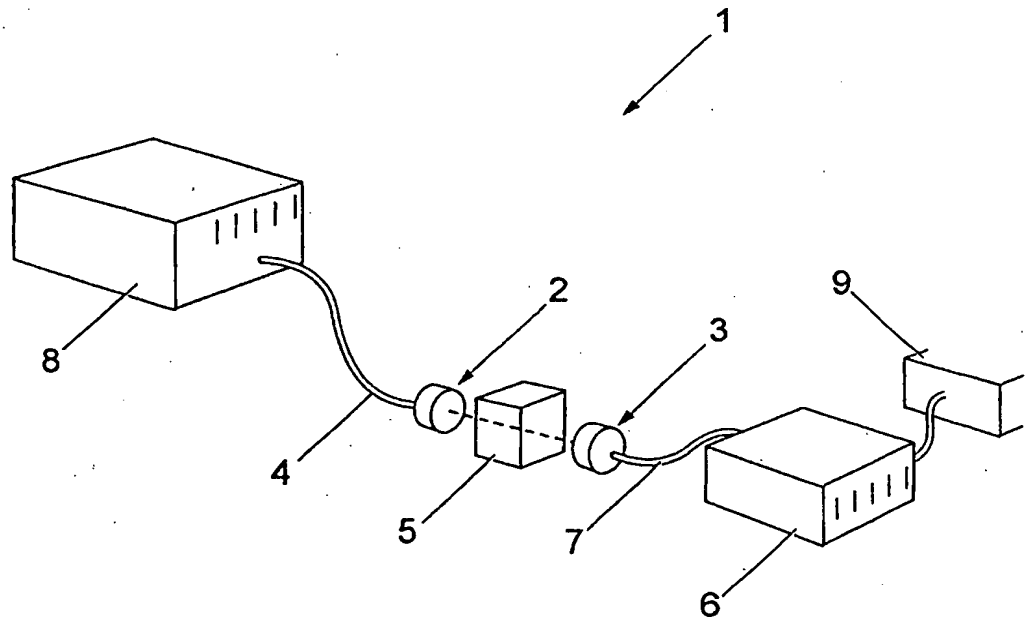

(57) Abstract

Spectrophotometrical apparatus for use in *in–vivo* conditions is provided. The apparatus includes a fibre–optics based device (11 having collecting fibres (11a) and emitting fibres (11b), computational apparatus and an NIR light source. The emitting fibre–optic (11a emit NIR light and collecting fibre–optic (11b) are positioned to collect light reflected back from within a sample of tissue *in–vivo*. Selecte parameters from computational analysis of the data received from the collecting fibre–optic are displayed in real–time to enable detectic of the onset of, for example, ischaemia within a sample of soft tissue *in–vivo*.

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Apparatus for spectrophotometry and method of obtaining spectrophotometrical information

The present invention relates to apparatus for spectrophotometry and to spectrophotometrical techniques, particularly in *in-vivo* conditions. More particularly the invention relates to the use of these techniques and apparatus in tissue, especially in soft tissue and blood vessels, and in particular those of the heart.

Spectrophotometry is an important quantitative analytical method to assess the concentration of different substances in complex chemical compounds. Spectrophotometry is based on the known properties of electromagnetic waves in different media, like absorbance, transmission or reflectance, as described for example in the Lambert-Beer Law. The amount of light passing through a substance is dependent upon the wavelength, thus, infrared (IR), visible (VIS) and ultraviolet (UV) light have different characteristics for spectrophotometry.

IR-, VIS- and UV-light obey the geometric rules of the electromagnetic radiation and can technically be

transported in a carrier medium (i.e. optic fibres) to a target.

Spectrophotometry is a technique mainly used in laboratories. The compounds to be detected have to be prepared specifically and placed in the spectrophotometer. The illumination of the substance is either directly by light or by using light which is transported in fibre optic devices between the source and the target. Biological compounds, due to their complex nature, are often a subject of spectrophotometric evaluation. Since these substances have to be extracted from living organisms and placed in a spectrophotometer however, the measurements can be performed only *in vitro*. Nevertheless, most biological molecules show different properties under *in-vivo* and *in vitro* conditions, which sometimes can make the results of the *in vitro* spectrophotometry questionable.

Each substance has a specific chemical configuration, which can be identified by its absorbance profile in the electromagnetic spectrum. Some substances (for example proteins) have absorption spectra in lower wavelengths called ultraviolet, some (for example metal ions) in the visible part of the spectrum (for example between 400-700 nm) and some in the infrared or near infrared area. The near infrared (NIR) is the part of the spectrum with wavelengths just above the visible, typically beginning at 700-730nm wavelength. Blood contains a significant amount of haemoglobin, which is a protein with an iron-complex. It can carry different molecules like oxygen and/or carbon dioxide, leading to changes in blood colour (visible spectrum), also to differences in the NIR-absorption characteristics.

The visible changes in blood colour have widespread use.

in *in vitro* controls of oxygenation status of blood, However, based on very moderate penetration capacity of the visible light into blood or tissue, *in-vivo* use is strongly limited. In contrast, NIR has a penetration of approximately 60-80 mm into soft tissue, including blood. This property makes it suitable to reliably detect the conditions of the tissue metabolism.

Attempts to use spectrophotometrical analysis under *in-vivo* conditions have been made within the past two decades, mainly in monitoring of oxygen-dependent chromophores like haemoglobin or cytochromes. However, such attempts have used bulky devices such as optodes, which are pads attached to the surface of the body over the area to be sampled. Such instruments are thus not overly suited for *in-vivo* monitoring of internal organs such as the heart and its surrounding tissue due to their size and the required geometry of the apparatus: as optodes work always in a dual configuration with one emitting light and the other collecting light which has been transmitted through the tissue sample a geometrical separation of illuminating and detecting optodes is necessary and can be difficult or impossible to achieve in *in-vivo* conditions. Despite the various possibilities of using NIR in clinical or laboratory medicine, therefore, widespread application has been limited.

Every tissue in an organism is perfused by blood to maintain its natural function. In case of a reduction of blood flow into the tissue, a situation called "ischaemia" arises, characterized by a lack of tissue nutrients, including oxygen and a congestion of metabolic waste products. If uncorrected, this situation can lead to irreversible tissue damage and finally to tissue death, called "infarction".

Infarction can virtually take place in every tissue: however, different tissues have different tolerances to ischaemic conditions, particularly the heart muscle (myocardium) shows a high sensitivity to ischaemia. Clinically, the ischaemic status of the heart is expressed by the term "angina pectoris", which, if untreated, may lead to a "myocardial infarction". Depending on the size and location of the infarctional area of the heart muscle, cardiac function may deteriorate to a level, that the patient is not able to survive.

To prevent a myocardial infarction, it is of significant clinical importance to detect ischaemia in an early phase, where therapeutic interventions have a good chance for success.

The heart, being a biological pump has three different functional properties:

I) mechanical : pumping function, building pressure and generating blood flow;
II) electrical : generating electrical current, facilitating coordinated pumping function;
III) chemical : utilize substrates to produce the energy to drive the pump.

Pressure-flow relationships as the outcome of the mechanical function are a crude tool in the detection of ischaemia, since they usually present clinically recognizable changes once the infarction has already occurred.

The clinical tool to observe the electrical activity of the heart is the electrocardiogram (ECG). Although ECG is a sensitive instrument, there are several clinical

conditions where this approach does not allow a timely detection of myocardial ischaemia, especially at the moment where a therapeutic intervention could prevent infarction.

Myocardial infarction is accompanied by damage of the cellular membrane, releasing different intracellular chemical substances into the blood, for example the enzyme creatinephosphokinase (CPK/CK-MB) or troponine. These substances can be identified by use of blood samples as a delayed response several hours after the infarction. For detection of myocardial ischaemia in prevention of infarction, it is evident that the use of this technique is fairly limited. Where the chemical changes in the early phase of ischaemia are concerned, the aforementioned conventional method is not successful to detect them.

The present invention seeks to provide apparatus for spectrophotometry of a tissue sample and a method of obtaining spectrophotometrical information relating to at least one internal condition of the tissue sample. In particular, the apparatus enables changes in soft tissue and body fluids, preferably blood, to be detected under *in-vivo* conditions. Further, the invention seeks to provide real-time, on-line detection of early phase chemical changes of ischaemic myocardium by NIR-technology in blood coming directly from the tissue.

According to a first aspect of the invention, there is provided a fibre-optic device for determining at least one internal condition of a tissue sample, the device including a catheter having: a fibre optic bundle with at least one light emitting fibre optic; at least one light collecting fibre optic; and a probe head provided

at a distal end of said catheter. The said at least one emitting fibre optic is capable of emitting light at said distal end of the catheter and said at least one collecting fibre optic is capable of collecting light at said distal end which has been emitted by said emitting fibre optic and subsequently reflected from the tissue sample.

The fibre optics may be provided coaxially within the catheter. The probe-head 18 may further include a shield lens. Preferably, the device is used in *in-vivo* conditions. Preferably, the wavelength of light is at least partially in the NIR region of the electromagnetic spectrum.

According to a second aspect of the invention, a spectrophotometrical apparatus for determining at least one internal condition of a tissue sample is provided. The apparatus includes the fibre optics-based device according to the first aspect of the invention and further includes an opto-electric signal conversion means which receives light signals collected by the said at least one light collecting fibre optic of the device; a computer connected to said opto-electric signal conversion means; and a light source connected to the said at least one light emitting fibre optic of the device. The said at least one light emitting fibre optic is capable of emitting light from the light source and the said collected reflected light signals are received by the opto-electric signal conversion means and are converted into electrical signals which are received by the computer. The computer performs analysis on said received electrical signals in order to determine at least one selected parameter related to said at least one internal condition of the sample of tissue.

Preferably, the apparatus further includes display means to display said at least one selected parameter. Preferably, said at least one selected parameter is determined and displayed in real-time. Preferably, said at least one selected parameter is obtained from light reflected within the sample of tissue *in-vivo*.

According to a third aspect of the invention, there is provided a method of determining an internal condition of a sample of tissue in *in-vivo* which comprises: illuminating a sample of tissue *in-vivo* with NIR light; collecting light reflected from within the sample of tissue *in-vivo*; converting said collected light signals into suitable electrical signals; inputting said electrical signals into a computer; analysing the electrical signals relating to the light reflected within the sample of tissue *in-vivo* obtain at least one selected parameter relating to the conditions within the sample of tissue *in-vivo*.

Preferably, the said at least one selected parameter is capable of indicating the level of ischaemia in the sample of tissue *in-vivo*.

According to a fourth aspect of the invention, there is provided a use of the device according to the first aspect of the invention or the apparatus according to the second aspect of the invention capable of detecting an internal condition of a sample of tissue *in-vivo*.

Preferably, ischaemic conditions are capable of being detected. Further, the use of the device or apparatus may be in a method capable of treating ischaemic conditions in a sample of tissue *in-vivo*. Further, the use of the device or apparatus may be in a method capable of preventing tissue infarction in a sample of

tissue *in-vivo*.

Preferably, the probe-head is inserted into the sample so that light is reflected and transported without encountering any major internal change in the light-propagating medium, for example a tissue-air boundary surface, so that the light is transmitted and reflected in a single tissue medium.

The amount of radiation collected by the collecting fibre-optic depends on the fraction of radiation emitted by the illuminating fibre-optic which is reflected back towards the collecting fibre-optic. The level of light reflected back towards the collecting fibre-optic is dependent on its wavelength and on the condition of the sample, for example its refractive index, absorption properties and any inhomogeneity which are present.

The fraction of light which is reflected will depend on the amount the transmitted light penetrates into the sample before it is absorbed or undergoes a deviation in its path (the path-length x). Only light which is 'back-scattered' or reflected back towards the collecting fibre-optic is analysed to determine the sample's condition.

Light which is transmitted to a depth 'x' in the sample before being reflected back towards the collecting probe can therefore provide an indication of the internal sample conditions to that depth 'x'. It should be clarified that if the probe is not inserted directly into the sample of tissue *in-vivo*, but if it instead merely faces a surface of the sample then, as light propagates from the probe to the sample it will encounter a surface boundary (for example air/tissue)

and this can generate the dominant part of the reflected light spectrum. Similarly, any internal boundary surfaces (for example, such as a bone/soft tissue boundary) would generate a strong reflection spectrum. Light which does, however, penetrate the sample's interior and which is then reflected back towards the probe would have to re-cross this surface boundary (i.e., tissue/air) before it could be collected by the collecting fibre-optic of the probe.

Preferably, therefore, the probe is inserted into the sample of tissue *in-vivo* and light reflected internally within the sample is collected. The term "transflectance" is used to refer to the light transmitted to various depths within the tissue which undergoes at least one scattering/reflection so that it is reflected back towards the probe's collecting fibre-optic.

The spectra of the "transflectance" is what is analysed to determine the internal conditions of the tissue sample. The internal conditions in the sample of tissue *in-vivo*, for example the level of oxygenation in blood/heart tissue, affects the "transflectance" by affecting how the light transmitted into the tissue propagates; for example, the internal reflection, absorption and transmission characteristics of the tissue may change.

It is the light which has propagated to various depths into the sampled area of *in-vivo* tissue before being reflected, or "back-scattered", termed herein the "transflectance" on which the detection of ischaemia largely depends. The onset of ischaemia is preferably detected by illuminating the tissue to be sampled using radiation in the visible and/or NIR waveband(s) and

more preferably using NIR radiation which lies in the 700-850 nm waveband. By selecting suitable wavelengths for the illuminating radiation, tissue can be sampled by collecting light which has penetrated preferably at least 60 mm before being reflected.

The collecting fibre-optic in the probe collects the reflected light and the optical signal is converted into electronic form by a suitable opto-electronic signal converter. The electronic signals are then inputted into a computer running a suitable program so that the program can be used to analyse the spectra of the transflectance. Preferably, the spectral analysis is performed using suitable computer software algorithms in real-time. Preferably, the level of ischaemia is displayed using suitable selected parameters in real-time on a suitable display means. This enables dynamic assessment of the level of for example, ischaemia or blood and/or tissue oxygenation to be made, for example, by a person observing the display.

Preferably the catheter can be introduced into the coronary sinus which is the main collecting vessel of venous blood coming from the myocardium. More preferably, the catheter is capable of being positioned within a coronary sinus and/or a right atrium of a heart directly in front of the coronary sinus.

Embodiments of the present invention will now be described, by way of example only, with reference to the accompanying drawings, in which:-

Fig. 1 is a sketch of a conventional apparatus for *in-vivo* spectrophotometry;

Fig. 2 is a sketch of a catheter apparatus according to the invention;

Figs. 3A and 3B are sketches of a probe-head according to the invention facing and inserted into a sample of soft tissue *in-vivo* respectively;

Fig. 4 is a sketch which shows how light is reflected internally within a tissue sample back towards the probe;

Fig. 5 is a sketch illustrating in more detail the catheter of Figs 3A, 3B and 4;

Fig. 6A is an end-on view of a probe in an embodiment of the invention; and

Fig. 6B is an end-on view of a probe in an alternative embodiment of the invention.

Fig. 7 is a sketch showing characteristic graphs of oxygenated or deoxygenated haemoglobin obtained in a specific example of a method of determining an internal condition of a sample of soft tissue according to an embodiment of the invention.

Fig. 8 is a sketch of a fibre-optic probe used in an embodiment of the invention.

Fig. 9 is a sketch showing a characteristic J-shaped curve of Hb/HbO_2 obtained from an arterial sample in a method of determining an internal condition of a sample of soft tissue according to an embodiment of the invention.

Fig. 10 is a sketch illustrating changes of coronary

sinus, arterial and peripheral venous system NIR-spectra.

Referring to the drawings, Fig 1 illustrates an apparatus 1 used in previous attempts to perform spectrophotometrical analysis under *in-vivo* conditions. For example, apparatus 1 has been used to monitor oxygen-dependent chromophores like haemoglobin or cytochromes. For these measurements, near infrared light (NIR) was used due to good tissue penetration (60-80 mm). The device 1 which is illustrated is based on the "optode" technique: optodes 2, 3 are pads to be arranged opposite each other with a tissue sample to be analysed in between. The optodes are geometrically arranged to emit light through the sample and to collect NIR light which has been transmitted through the sample. The NIR light is generated by a light source 8, for example laser diodes, and is carried to the tissue sample 5 via a fibre-optic cable 4. The light emerging from the tissue 5 is returned to a photodetector 6 through another fibre-optic cable 7. The light is suitably amplified and converted into an electrical signal, e.g. by a photomultiplier. Both signal analysis and data processing are performed by a computer 9 as illustrated in Figure 1.

Fig. 2 illustrates a fibre-optics device 10 according to the invention. The device 10 includes at least two fibre-optics 11a and 11b, which are normally groups of optical of which one group 11a transmits light to provide illumination and the other group 11b collects light. In use, the illuminating fibre-optic 11a is connected via a fibre-optic coupling interface 12a to a light-source (not shown) and the collecting fibre-optic 11b is connected via a fibre-optic coupling interface 12b to suitable opto-electric signal conversion means

(not shown), for example a photodetector and photomultiplier. The optical signals provided by the device 10 are thus converted into electric signals which can be suitably analysed, for example by a computer running an appropriate software package.

The fibre-optics 11a, 11b are arranged in a single bundle 19 at some point 14, preferably so that their fibres are formed into a suitably concentric array, for example so that the emitting fibres 11a axially surround the collecting fibres 11b. Other array configurations can also be provided in alternative embodiments. The bundle of fibre-optics 19 is then run along the interior of the catheter 17 and forms probe-head 18 (see Figs. 3A to 5) at the distal end of the catheter. Positioning the catheter 17 under *in-vivo* conditions may be facilitated for example, by providing a suitable handle 16 such as is shown in Fig. 2.

In use of the device 10, the illuminating fibre-optic 11a is connected to a light source (not shown) such as, for example, a laser diode. The radiation emitted from the illuminating fibre-optic 11a is selected to be mainly NIR light and/or visible light.

The rationale behind the use of the catheter 17 is a single location illumination/detection principle. As Figs 4 and 5 illustrate, there is no pre-determined path-length for the reflectance; i.e. undeviated light transmitted in the sample of tissue *in-vivo* is not collected. In the absence of any internal tissue structural change such as a soft tissue/bone boundary, and if unabsorbed, the emitted light could potentially have an unlimited path-length within the tissue sample. However, providing sufficient internal scattering/reflection occurs, a strong enough

internally reflected signal can be collected by the collecting fibre-optic 11b. This internally reflected signal is the "transflectance".

The depth to which the incident radiation can penetrate the tissue before being reflected is wavelength dependent and is affected by the condition of the tissue, in particular the level of inhomogeneity in the tissue structure. Inhomogeneity can provide scattering centres which can reflect light back towards the collecting fibre-optic 11b of the fibre-optic device 10. For example, blood corpuscles can reflect light back towards the collecting fibre-optic 11b if the fibre-optic device 10 is inserted into vein.

The collecting fibre-optic 11b receives light which has been reflected, i.e., light which has been emitted from the illuminating fibre-optic 11b towards the sample of soft tissue *in-vivo* and whose initial path has been deviated by substantially 180° after undergoing at least one reflection/scattering.

The collected light signals are suitably converted and amplified into electrical signals, for example by a conventional opto-electrical signal converter. The electrical signals are then supplied to a computer running a computational package. The computer may contain hardware dedicated to optimise processing of the received data representing the collected light signals, or may be alternatively a conventional computer with standard processing means, memory means, and I/O means. Visual display means are connected to said computer to display at least one selected parameter extracted from the received data by the computational package.

The computational package processes the received data inputted into the computer and performs a suitable data analysis to enable at least one selected parameter related to the condition of the sample of tissue *in-vivo* to be obtained.

Inserting the catheter 17 into a sample of tissue *in-vivo* enables information to be extracted from the spectrum collected which is capable of indicating the internal conditions of the sample of tissue *in-vivo*. The term "sample of tissue *in-vivo*" refers to the *in-vivo* region of the tissue (for example soft tissue, body fluid and especially blood) through which light emitted by the emitting fibre-optic 11b can penetrate and from which light can be reflected back to the probe's collecting fibre-optic 11a.

Providing the computational power and algorithmic structure of the data-analysis performed permits, the required information relating to at least one internal condition of the tissue sample can be displayed and monitored in real-time on the display means which receives the data to be displayed from the computer. The invention thus provides a means to detect the onset of any changes in the tissue condition which can be analyses at the NIR wavelengths, for example, ischaemia.

Figs. 3A to 4 are sketches which provides a cross-sectional illustration of the catheter 17 according to an embodiment of the invention. The probe-head 18 is an NIR-probe which is tissue compatible. The cross-section of the catheter 17 is based on the two groups of concentric fibre-optic 11a and 11b being arranged coaxially, for example, with the emitting fibre-optic 11a surrounding the collecting fibre-optic 11b such as

is sketched diagrammatically in Fig 6A.

The illuminating fibre-optic 11a transports the NIR-light (L_I) from the light source along the catheter 17 to the probe-head 18. In Fig. 3A, the probe-head 18 is not inserted into the tissue to be sampled and light (L_R) reflected from the tissue surface will provide the dominant part of the collected light. Only a very small fraction of light which penetrates the tissue to a depth x_1 is likely to be reflected (L_{TR}) and to reemerge at the tissue/air boundary and be collected as light (L_{TRT}).

If the probe is used as shown in Fig. 3B, which is the preferred mode of use according to an embodiment of the invention, incident light (L_E) is emitted through the probe head 18 directly into a tissue sample 20 *in-vivo*. Inserting the probe 18 into the tissue 20 enables the proportion of the light collected L_{TR} (which comprises light L_T which has penetrated to depths x_1, x_2 before undergoing at least one reflection) to be higher than if the probe were simply to face the tissue 20 surface as was shown in Fig. 3A. Obviously, the detection technique of the invention relating to determining at least one internal condition of the tissue sample relies on at least some of the emitted light undergoing internal reflection and back-scattering, such as is illustrated in Fig. 4.

Fig. 4 sketches how the emitted light (L_E) is transmitted into the tissue. The transmitted light rays L_T can be scattered (L_S) or reflected (L_R) by inhomogeneity within a tissue sample 20. In the absence of any internal scattering or absorption, the path-length of the NIR-light transmitted L_T into the tissue could potentially be infinite ($x \rightarrow$ infinity)

and no light L_c could then be collected by the collecting fibre-optic 11b.

The fibre-optic device 10 thus provides a means to obtain spectral information which can be analysed to determine certain selected characteristics of the tissue 20 sampled. For example, the transmitted NIR-light through the tissue is partly absorbed according to the specific absorption spectrum. By collecting the reflected part of the NIR-light, the absorption spectrum of the tissue can be obtained and monitored. Dynamic changes in the absorption spectrum can then be obtained by suitable computational means and statistical software packages.

In Fig. 5, a specific embodiment of the device 10 is shown in use. The device 10 includes a myocardial spectrophotometry (MSP) 17 catheter which is positioned like a regular central venous catheter. The probe-head 18 at the catheter tip is preferably located in the coronary sinus 30 to monitor myocardial ischaemic conditions. Positioning of the catheter 17 can be controlled using fluoroscopy and/or echocardiography. The catheter 17 is connected to a suitable NIR light source and a spectrophotometer (not shown) to enable the detection of ischaemia. If organs other than the heart are to be monitored for ischaemia, the catheter tip 18 should be placed in the collecting vein of the organ.

Ischaemic conditions of the myocardium lead to significant changes in the NIR-absorption spectra of the blood originating from the myocardium, including the oxygenation status. Blood typically displays peaks at the upper part of the visible spectrum. For

example, peaks at 570-580 nm and 615-630 nm, and also at 760-775 nm and at 800-850 nm. The time evolution of the blood spectrum can be monitored and preferably the evolution of at least one of the above four peaks is observed. The light source provides light over at least one of the above wavebands, and preferably over all four. Suitable tuning means may be provided to selectively control the wavelengths emitted by the light emitting fibre-optic 11b.

The size and shape of the spectral peaks obtained in the above regions of the NIR and visible spectrum change significantly and consistently if the myocardial workload changes. These patterns are reproducible under comparable ischaemic conditions of the myocardial tissue. Even slight differences in myocardial metabolism lead to significant changes of the NIR-absorption curves.

To ensure reliable spectra of the "transflectance" for the purposes of real-time analysis, it is sufficient to illuminate a tissue area with sufficiently intense light to achieve there desired level of penetration given the expected absorption properties of the light in the Visible, NIR and NNIR wavebands.

In the clinical use, this technology offers the option of detecting ischaemia of the myocardium at an early enough phase to enable intervention (for example, pharmacological, surgical, and/or cardiologic intervention) to be able to prevent the development of an infarction. This technology also allows a continuous real-time/on line monitoring of the myocardial perfusion status.

One embodiment of the invention provides a fibre-optic

catheter 17 for delivery of two or more distinct wavelengths λ_1, λ_2 of light to a sample, preferably blood, though it should be clear that the number of interrogation wavelengths, the size and shape of the sampling probe head and the means for transmitting the light to and from the sample can be varied to meet particular needs and applications. For instance, the apparatus can include a single or multiple wavelength illumination source, a wavelength specific detector array, and a power source.

A suitable illumination source is selected to illuminate a sample of tissue *in-vivo* at the selected wavelengths via the fibre optic bundle 19. The system is set up to detect visible and near infrared absorption and a suitable light source is a tungsten-halogen bulb in a quartz envelope to provide light in the desired NIR wavelength range.

In one embodiment of the invention, the apparatus included an NIR analyser fitted with a tungsten halogen lamp, an NNIR grating (600nm to 1200nm) and a lead sulphide fibre-optic detector. This used a conventional oscillating scanning monochromator which provided around five complete spectral scans per second. A spectral acquisition consisted of the average of 30 scans and took approximately 20 seconds per acquisition.

An alternative embodiment the apparatus included a Photo Diode Array (PDA) Optical Spectrograph Card which was located within a PC controller. A separate module contained a tungsten-halogen light source and its power supply. The wavelength range was 380 nm to 1100 nm. Utilising Charge Coupled Device (CCD) technology, this enabled the spectral domain to be scanned as quickly as

every 5 milliseconds.

In yet another alternative embodiment, the apparatus included a CCD array spectrophotometer with 2048 pixels and a wavelength range of 300 to 1100 nm. A stabilised tungsten-halogen light source was utilised and the spectrometer, light source and fibre-optic device 10 were all fitted with SMA couplings. Spectral acquisition times were of the order of 2 seconds per sample.

In all of the above embodiments, suitable software was provided so that appropriate data acquisition, graphing and data manipulation was possible. The CCD devices enabled rapid spectral acquisition times, up to the order of 2 seconds per sample which can be compared to 20 seconds per sample using the conventional spectrophotometer. This enabled 10 separate scans to be made for a sample and the average obtained.

The data acquisition procedure consists of collecting reference spectra to enable a dark current correction, and a white reflector correction. A "normal" sample spectra consists of the average of 20 spectra, corrected for dark and reference backgrounds. Optionally, multiple samples at the same time point were taken and averaged for data analysis. During *in-vivo* operation, it was not possible to retake updated dark or reference backgrounds. Data obtains *in-vivo* can show some signs of CCD drift over long periods of data collection and dark corrections against this should be made at regular intervals of time, for example, hourly.

The collected light exhibited changes in the wavelength range of 600 to 1100 nm as the internal conditions of

the tissue sampled, for example blood concentration and constituents, varied. Multi-variate analysis enable correlations to with selected parameters relating to oxygen content and pressure, CO_2 content and pressure, haemoglobin content, and pH. Even spectra obtained without any subsequent mathematical transformation could be observed to change significantly with changing states of ischaemia.

The fibre optic bundle 19 of the fibre-optic device 10 used in conjunction with the spectrophotometric apparatus described in the above embodiments is made up basically of a bundle of optical fibres 11a, 11b. The afferent (collected) and efferent (emitted) optical signals are carried by separate optical fibres 11a, 11b, within the bundle 19. The diameter of the bundle 19 is preferably about 0.1 mm to 3 mm and the bundle 19 contains several emitting fibres 11a and collecting fibres 11b, for example 75 collecting and 75 emitting fibres each of whose diameters is approximately 200 μm.

The fibre-optics 11a, 11b terminate in the fibre optic probe 18 located at the tip of the catheter 17, such as Figs 4 and 5 illustrate. The probe 18 illustrated in Figs. 4 and 5 includes a shield lens 25 at the distal end of the probe-head 18 so that non-contact probing may be achieved, facilitating examination of areas within a blood or tissue sample. Light from the light source is fed through suitable coupling interface 12a into an input leg of the efferent (emitting) fibre-optic 11a of the optic fibre bundle 19. The light entering the fibre optic bundle 19 emerges at the distal end of the fibre, e.g. at probe head 18, and is conducted out of the probe head 18 through probe head shield 25 so as to penetrate the sample of tissue *in-vivo*.

The shield 25 may be in the form of a glass, fused silica, sapphire or other transparent member. The shield 25 may be flat, spherical or lens shaped. The periphery of the shield 25 is bonded to the end of the probe wall 26.

In one embodiment of the invention, the shield is selected to provide a means of focusing the emitted light and/or the collected light. Such focusing can be used to control the fraction of light emitted which is collected and/or affect the extent to which emitted light penetrates the tissue sample.

The method used to obtain real-time information on the dynamic changes of the spectral data collected by the probe 18 in a preferred use of the device 10 will now be described in more detail. The spectral data was obtained from an Indium gallium arsenide photodiode array spectrometer. The spectrometer was fitted with quartz fibre optic core catheter which was inserted in various blood vessels in the heart and different areas of the body. Spectra were obtained from blood within the coronary sinus and from what are termed reference points such as the arterial blood system.

The spectral data was treated using a commercially available Multivariate statistical package called Unscrambler (provided by Camo Norway). The spectra were pre-processed in numerous ways for example transmission, reflectance, first and second derivative or combination of the above. Other possibilities for pre-processing include light scattering reduction techniques such as multiplicative scatter correction in association with the stated pre-processing methods.

Using the Chemometric technique of Partial Least

Squares modelling correlations in both coronary sinus blood and arterial blood can be obtained for the following parameters: Haemoglobin content, pCO_2 content, Oxygen content, pO_2 content, and SO_2 content.

Obviously other parameters can be extracted using suitable analysis routines. Once the desired parameters have been extracted, the relevant information is displayed so that any changes can be monitored by a user. Ideally, the changes are up-dated within a time-scale of the order of seconds, preferably less than 10 seconds, more preferably less than 3 seconds to enable a user to monitor any changes in real-time. Real-time monitoring can, for example, be achieved on a time scale less than 10 seconds where data is acquired on time-scales of the order of 2 seconds such as can be obtained, for example, using a CCD spectrophotometer.

In particular, if the information obtained indicates that certain changes in the tissue condition are occurring which may be prevented by suitable therapeutic intervention (for example application of a suitable medicament to the sampled region), the catheter 17 can be further provided with means to intervene therapeutically, e.g. apply such a medicament. Fig. 6B illustrates an embodiment of the invention in which such means to intervene therapeutically comprise an aperture or internal pipeline 50 provided within in the catheter. The aperture 50 is provided within the fibre optics bundle 19 so that a medicament can be applied to the sample of tissue *in-vivo* 20 and its effect subsequently monitored.

Ideally, the aperture is provided centrally within the

catheter 17 and may provide a means for monitoring other conditions within the sample of tissue *in-vivo* 20, for example, pressure measurement. Such additional monitoring means may further include means to provide therapeutic intervention, i.e., drug administration.

Use of the device 10 need not be restricted to an in-vivo tissue sample. For example, reliable information can be obtained by inserting the device 10 into an *in-vitro* sample.

A specific example of use of the device 10 and apparatus relating to continuous real-time monitoring of myocardial ischemia by a new fibre-optic NIR-catheter is described below.

Near InfraRed Spectroscopy (NIRS) is a relatively new technique to assess concentration changes of different substances in the living tissue. The main application field is the detection of oxyhaemoglobin and deoxyhaemoglobin, as well as the redox state of cellular mitochondrial Cytochrome aa3. The tissue penetration of NIR is up to 6-10 cm, making it a noninvasive, suitable monitoring tool.

NIRS measurements take place between 650-1200 nm. Some devices are capable of extend the lower end of the spectrum to the visible wavelengths, down to 550-570 nm. The characteristic graphs of oxygenated (HbO_2) or deoxygenated (Hb) haemoglobin are shown in Fig. 7.

Both graphs are reversible and can change dependent upon the O_2 saturation. In humans, the normal *venous* blood has an average O2- saturation of 60-70% with a characteristic absorption peak at 760-780 nm, which becomes more pronounced if the haemoglobin oxygenation is reduced. The arterial haemoglobin with an

appropriate level of O_2-saturation, for example > 70%, shows a J-shaped smooth curve up to 1050 nm. These graphs have an isobestic point at 805-815 nm. However, as the level of O_2-saturation falls, peaks can appear and the spectrum evolves.

Currently, NIRS is a well established monitoring technique of the blood and tissue oxygenation. The monitoring of oxygen-dependent chromophores by NIRS needs a NIR-source (optode) for illumination and a fibre-optic bundle to transfer light to the tissue. The transmitted light is collected on a second optode and carried by another fibre-optic cable to a photomultiplier, which converts the light to an electric signal. Both signal and analysis and data processing are to be performed by a computer (as shown, for example, in Fig. 1).

The in vivo use of optodes *in* bloodstream is virtually impossible due to the too bulky configuration of these devices (for example, typical optode dimensions range from 1cm to 5cm) and thus locating an optode within most in-vivo tissue environments, for example, a blood-vessel is not feasible. Another problem is generated by the corpuscular elements of blood, which disturb the processing of signals. Therefore, blood NIR-measurements can only be carried out indirectly through a tissue or in-vitro, using collected tissue or blood samples.

In a specific example of obtaining blood NIR-measurements to detect the local differences of O2-saturation in the myocardial tissue, 12 pigs (domestic swines) were used to collect blood samples 21 from the coronary sinus and different locations of the circulatory system. The oxygenation of haemoglobin in

arterial, peripheral and coronary sinus blood could be detected. The measurement were performed by using the fibre-optic device 10 according to an embodiment of the invention in a glass tube directly after the collection of the blood samples (Fig. 8).

Coronary sinus blood samples showed different shapes of Hb/HbO_2 graphs than peripheral venous blood samples, displaying a more pronounced peak at 760-780 nm. Arterial samples showed always the characteristic J-shaped curve (Fig. 9).

To evaluate the changes in myocardial oxygen consumption and utilization in acute ischemia, the left anterior descending coronary artery (LAD) in 12 pigs was occluded by a string snare between the first and second diagonal branch, setting a large ischemic zone in the left ventricular anterior wall. The ischemia was very predominant in pigs due to the lack of collaterals. Both samples were collected in 15 min - periods from the carotid artery, femoral vein and the coronary sinus. The results indicated that, after beginning of the ischemia, the pig heart showed a rapid deoxygenation of the coronary sinus blood which led to significant changes of the coronary sinus NIR-spectra. The changes in the arterial and the peripheral venous system however were definitely not significant (Fig. 10).

The differences in the coronary sinus blood are expressed by a progredient increase of the Hb-peak at 760-780 nm. The impaired oxygenation after myocardial ischemia and infarction respectively could be seen in the Hb/HbO2-curves and reliably be detected, even if the ECG-changes were not always remarkable. In the pig experiment, the most important question was to obtain

the cause of the decrease in haemoglobin oxygenation. Since the ischemic myocardium is normally not perfused, the reduced O2 saturation in the coronary sinus blood could not be explained by the onset of the ischemia. The transient elevation of the O2-consumption was most probably generated by the increased activity of the perfused myocardium to maintain the cardiac output. This could also be seen in the sudden increase of the heart rate.

To compare the changes of pig haemoglobin during oxygenation/deoxygenation, with those in the human blood, a closed circuit using a paediatric extracorporeal circulation unit was assembled. The system had an oxygenator and an heat exchanger. The priming was approx. 600 mL. In this in-vitro-experiment, the possibility existed to increase or decrease the oxygen saturation at a constant temperature of 37°C. The probe was integrated into the circuit and an on-line NIR-scanning was performed. The oxygen saturation was detected in samples by a regular oxymeter to make a correlation with the NIR-curves possible. Since the blood was venous, low-O_2-levels were used initially and the saturation was increased gradually.

The results of this experiment were:

1) the specific coronary sinus-graphs were a direct result of the actual low O_2-saturation;

2) as soon as the O_2-saturation was higher than 50-60%, the graph became "arterial"; and

3) the normal venous O_2-concentration gave a slight peak at 760-770 nm, while a desaturation led to a

higher peak at this point.

In an in-vivo experiment, the selective cannulation of the coronary sinus seemed to be too risky for the patient at this stage. Therefore, the right atrium was double-cannulated and both the superior and inferior venae cavae were stringed to avoid the backflow from the peripheral circulation. A catheter was placed into the right atrium to collect blood samples from the coronary sinus. The measurements obtained virtually led to the same outcome as those with the selective cannulation of the coronary sinus.

The conclusion can be summarized as follows:

1) the in vivo-measurement in humans appears to show no significant difference between animal and in-vitro measurements;

2) the myocardial oxygen consumption appears to be capable of being reliably detected by placing a NIR-catheter directly into the coronary sinus or into a suitable position in the right atrium, directly in front of the coronary sinus; and

3) the protection grad of the myocardium during moderate hypothermia with intermittent aortic cross-clamping appears to be capable of being identified by the NIRS.

The results obtained in pigs and in human blood encourage use of NIRS as a real-time continuous on-line detection method for the myocardial perfusion status, particularly in patients with an acute ischemia. The differences in haemoglobin oxygenation curves, background by detectable hemodynamic alterations and

ECG-changes, lead to the conclusion that these measurements can be used as a suitable monitoring tool for acute myocardial infarction.

While several embodiments of the present invention have been described and illustrated, it will be apparent to those skilled in the art once given this disclosure that various modifications, changes, improvements and variations may be made without departing from the spirit or scope of this invention.

For example, alarm means may be provided so that in a case where ischaemic conditions are detected a suitable signal is generated to alert a person to such conditions. Further, automatic application of a medicament may be provided to intervene therapeutically in such a case.

The text of the accompanying abstract is incorporated herein by reference.

Claims

1. A fibre-optic device 10 for determining at least one internal condition of a tissue sample, the device 10 including a catheter 17 having:

a fibre optic bundle 19 with at least one light emitting fibre optic 11a; at least one light collecting fibre optic 11b; and a probe head 18 provided at a distal end of said catheter 17;

wherein said at least one emitting fibre optic 11a is capable of emitting light at said distal end of the catheter 17 and said at least one collecting fibre optic 11b is capable of collecting light at said distal end which has been emitted by said emitting fibre optic 11a and subsequently reflected from the tissue sample.

2. A device 10 as claimed in Claim 1, wherein the emitting and collecting fibre optics 11a, 11b are provided coaxially within the catheter 17.

3. A device 10 as claimed in any preceding claim, wherein the probe head 18 further includes a shield lens 25.

4. A device 10 as claimed in any preceding claim, wherein the device 10 is for use in *in-vivo* conditions.

5. A device 10 as claimed in any preceding claim, wherein the emitted light is reflected and collected substantially within one tissue medium *in-vivo*.

6. A device 10 as claimed in any one of claims 4 to 5, wherein said catheter 17 further includes additional non-optical means 50 to monitor at least one internal condition of a tissue sample *in-vivo*.

7. A device 10 as claimed in any one the preceeding claims wherein said catheter 17 further includes means to apply a medicament 50 to the sample of tissue *in-vivo*.

8. A device 10 as claimed in claim 7, wherein said medicament is for the therapeutic intervention of ischaemia.

9. A spectrophotometrical apparatus for determining at least one internal condition of a tissue sample, the apparatus including:

the fibre optics-based device 10 as claimed in any one preceding claim;

an opto-electric signal conversion means which receives light signals collected by the said at least one light collecting fibre optic 11b of the device 10;

a computer connected to said opto-electric signal conversion means; and

a light source connected to the said at least one light emitting fibre optic 11a of the device 10;

wherein said at least one light emitting fibre optic 11a is capable of emitting light from the light source and wherein said collected reflected light signals are received by the opto-electric signal conversion means and are converted into electrical signals which are received by the computer, and wherein the computer performs analysis on said received electrical signals in order to detect variations in at least one selected parameter related to said at least one internal condition of the sample of tissue.

10. Apparatus as claimed in claim 9, further including display means to display data relating to said at least one selected parameter.

11. Apparatus as claimed in either claim 9 or claim 10, wherein the data relating to said at least one selected parameter is obtained by performing analysis on data derived from light which has been reflected within the sample of tissue.

12. Apparatus as claimed in claim 11, wherein the at least one selected parameter relates to light reflected within a sample of tissue *in-vivo* and is capable of indicating the level of ischaemia in the sample of tissue *in-vivo*.

13. Apparatus as claimed in any one of Claims 11 to 12, wherein the at least one selected parameter relates to intraluminal blood Hb and/or HbO_2 and/or Cyt aa3 content.

14. Apparatus as claimed in any one of Claims 11 to 13, wherein at least one selected parameter is chosen from the group consisting of: Haemoglobin content, pCO_2 content, Oxygen content, pO_2 content and SO_2 content.

15. Apparatus as claimed in any one of Claims 12 to 14, wherein said at least one selected parameter is determined by performing analysis on data derived from the collected light signals in real-time and in which said display means displays in real-time any changes in said selected parameter so obtained.

16. A device 10 or apparatus as claimed in any one preceding claim, further comprising means to select the wavelengths of the light emitted by said at least one light emitting fibre optic 11a.

17. A device 10 or apparatus as claimed any one

preceding claim, wherein the light emitted at least partially occupies the NIR region of the electromagnetic spectrum.

18. A device 10 or apparatus as claimed in any one preceding claim, wherein the catheter 17 of the device 10 is capable of being positioned within a coronary sinus and/or a right atrium of a heart directly in front of the coronary sinus.

19. A method of determining an internal condition of a sample of tissue *in-vivo* comprising:-

illuminating a sample of tissue *in-vivo* with NIR light;

collecting light reflected from within the sample of tissue *in-vivo*;

converting said collected light signals into suitable electrical signals;

inputting said electrical signals into a computer;

analysing the electrical signals relating to the light reflected within the sample of tissue *in-vivo* obtain at least one selected parameter relating to the internal condition of the sample of tissue.

20. A method for detecting ischaemia as claimed in claim 19, wherein said at least one selected parameter is capable of indicating the level of ischaemia in the sample of tissue *in-vivo*.

21. Use of the device 10 or apparatus as claimed in any one of claims 1 to 19 capable of detecting an internal condition of a sample of tissue *in-vivo*.

22. Use of the device 10 or apparatus as claimed in Claim 21, wherein ischaemic conditions are capable of being detected within the sample of tissue *in-vivo*.

23. Use of the device 10 or apparatus as claimed in Claim 22, in a method capable of treating ischaemic conditions in a sample of tissue *in-vivo*.

24. Use of the device 10 or apparatus as claimed in any one of claims 21 to 23 in a method capable of preventing tissue infarction in a sample of tissue *in-vivo*.

25. A device 10, apparatus, method or use as claimed in any one preceding claim, wherein the sample of tissue is soft tissue.

26. A device 10, apparatus, method or use as claimed in any one preceding claim, wherein the sample of tissue is body fluid.

27. A device 10, apparatus, method or use as claimed in any one preceding claim, wherein the sample of tissue is blood.

28. A device 10, apparatus, method or use as claimed in any one preceding claim, wherein the sample of tissue is heart tissue.

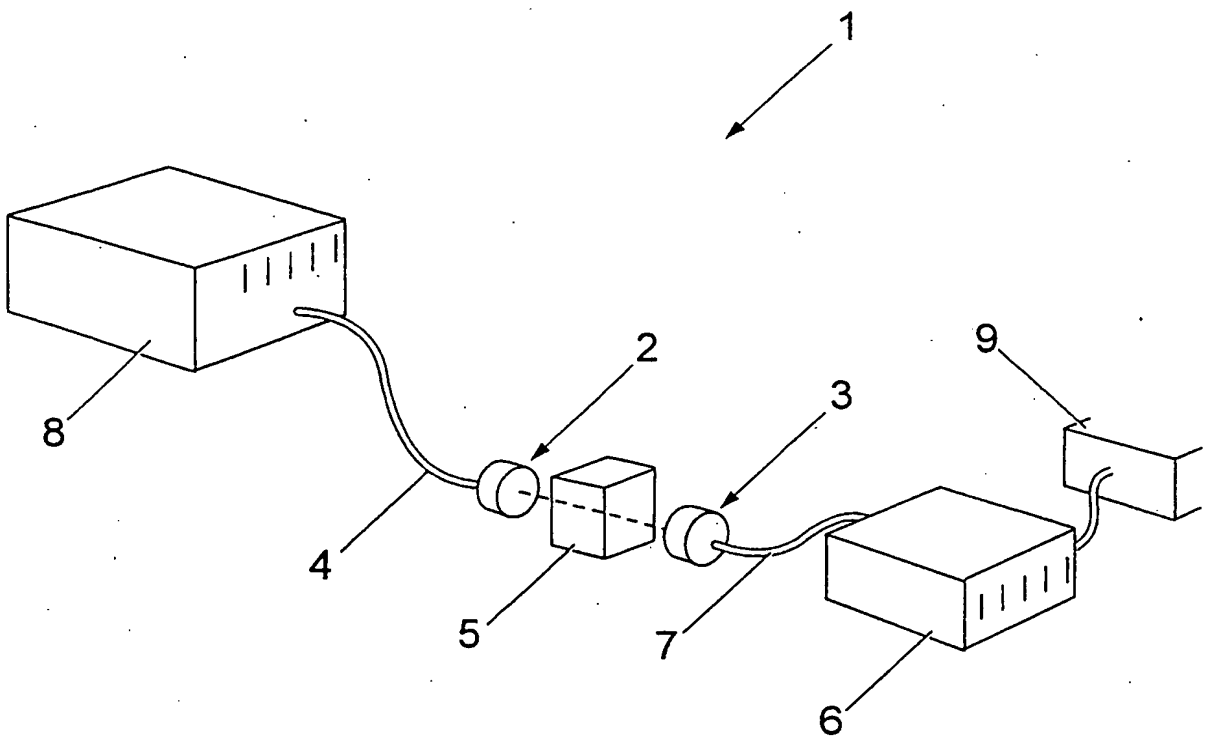

Fig. 1

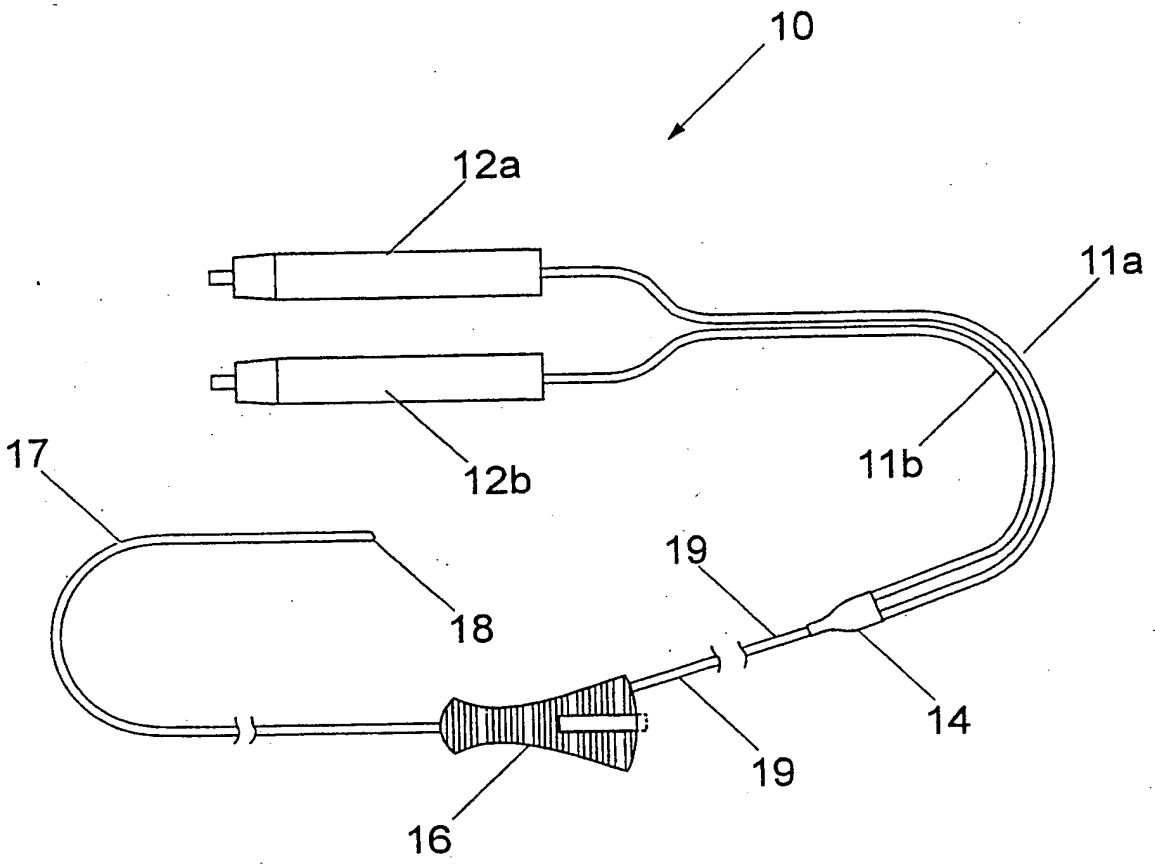

Fig. 2

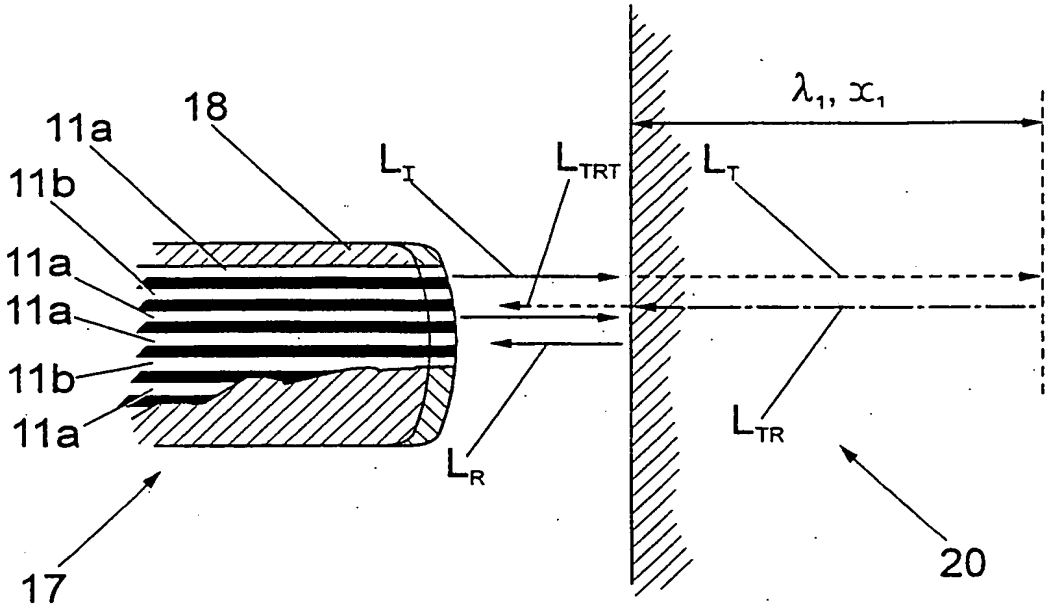
18
11a
11b
11a
11a
11b
11a
17
L_I
L_{TRT}
L_R
λ_1, x_1
L_T
L_{TR}
20

Fig. 3a

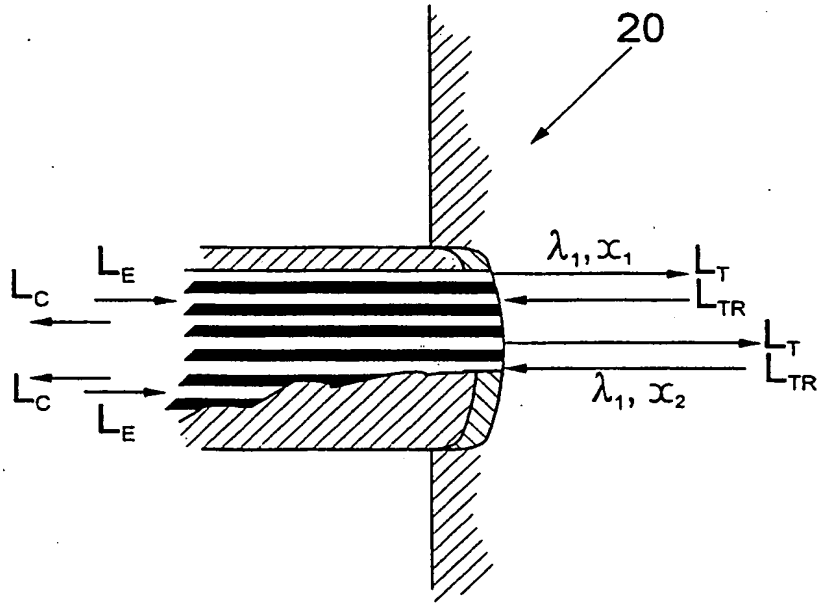
20
L_E
L_C
L_C
L_E
λ_1, x_1
L_T
L_{TR}
L_T
L_{TR}
λ_1, x_2

Fig. 3b

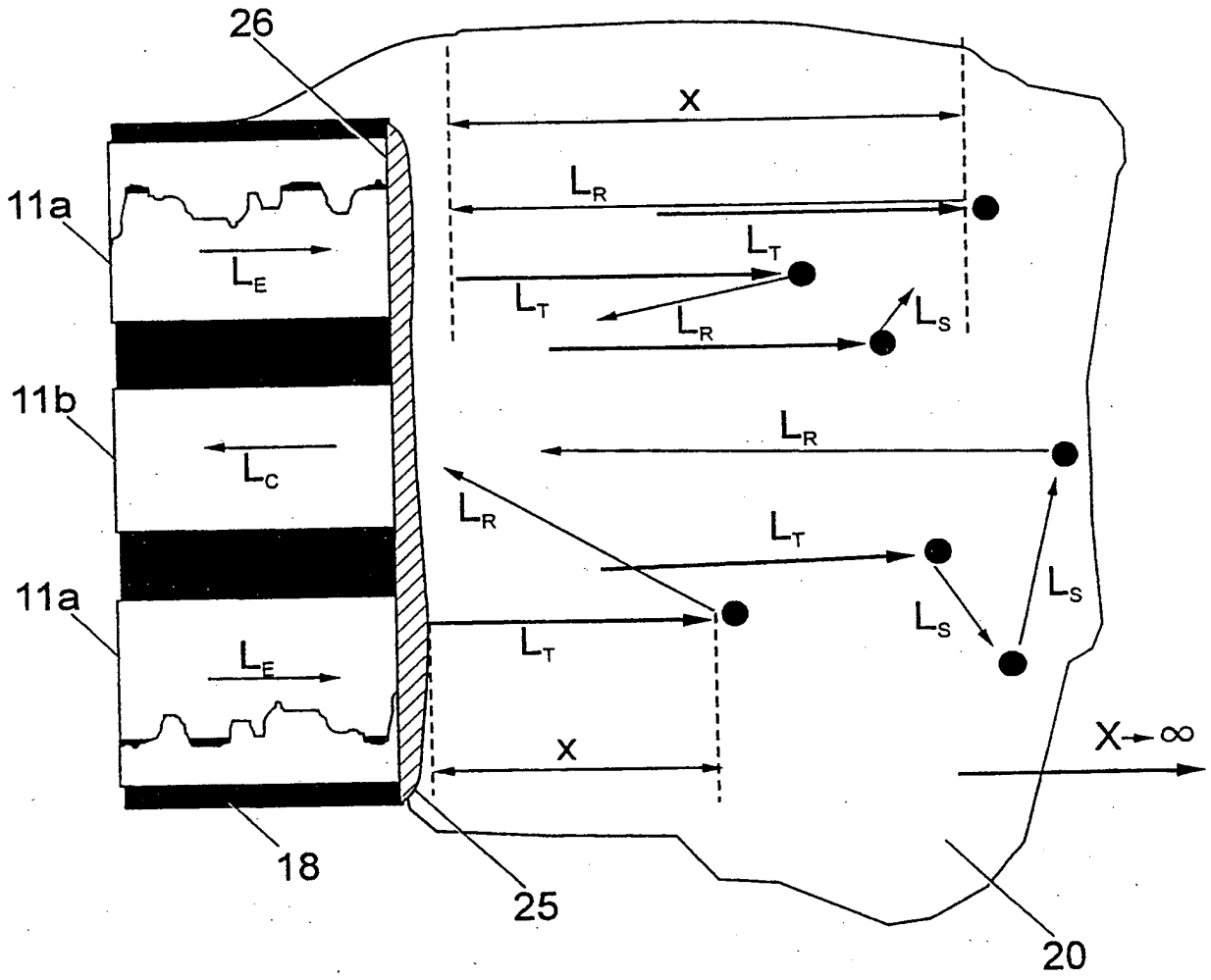

Fig. 4

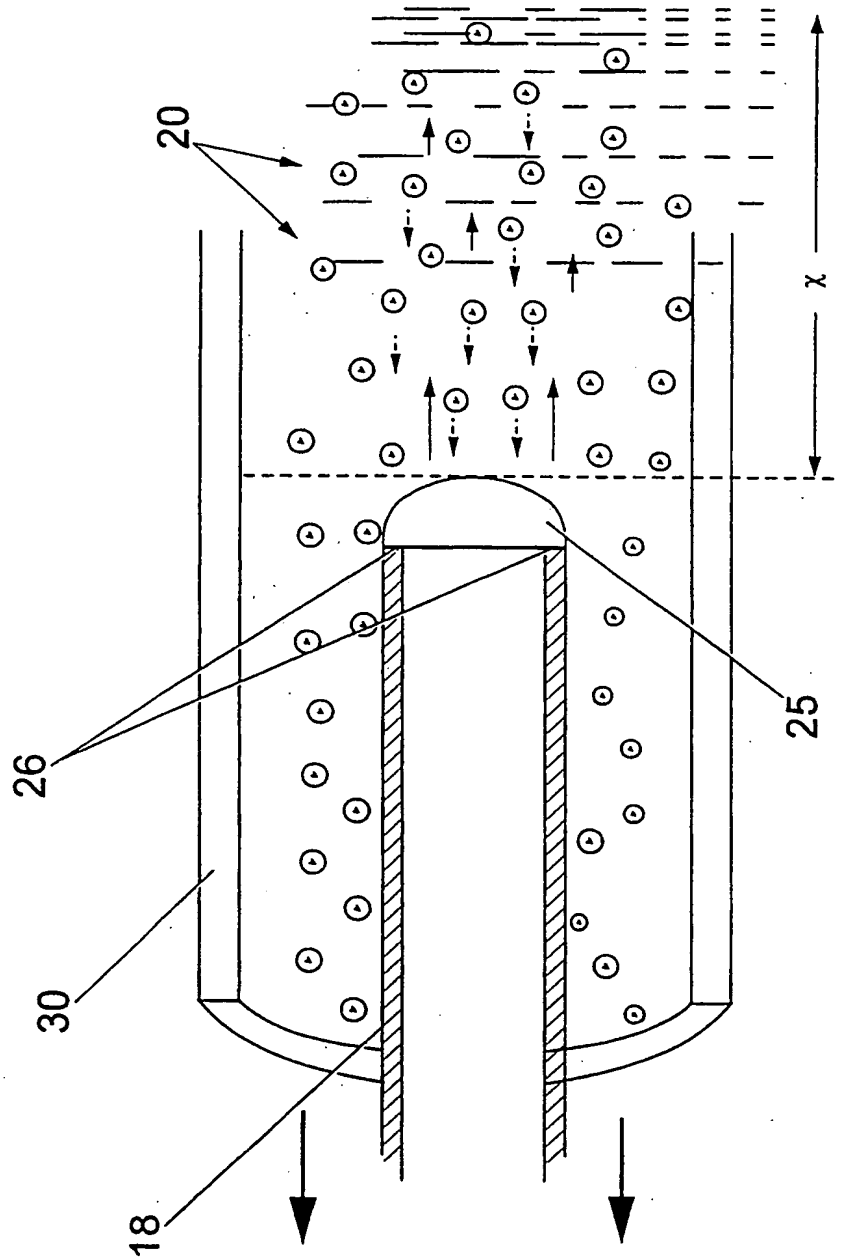

Fig. 5

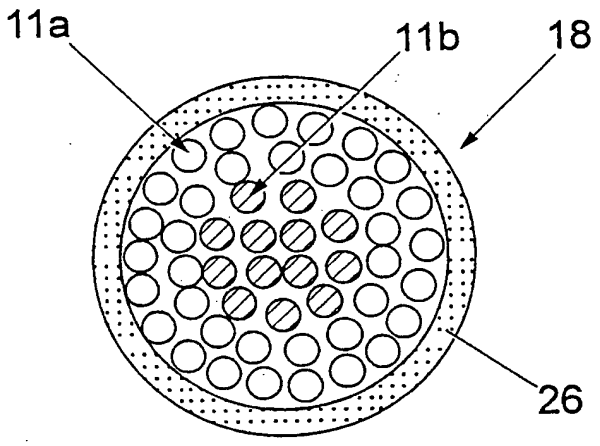

Fig. 6a

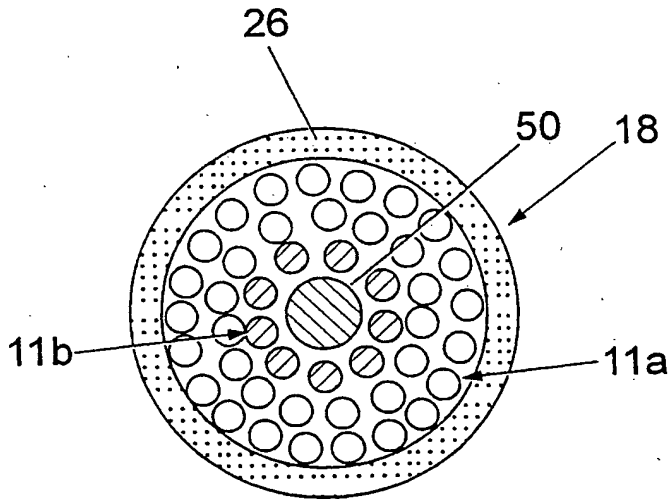

Fig. 6b

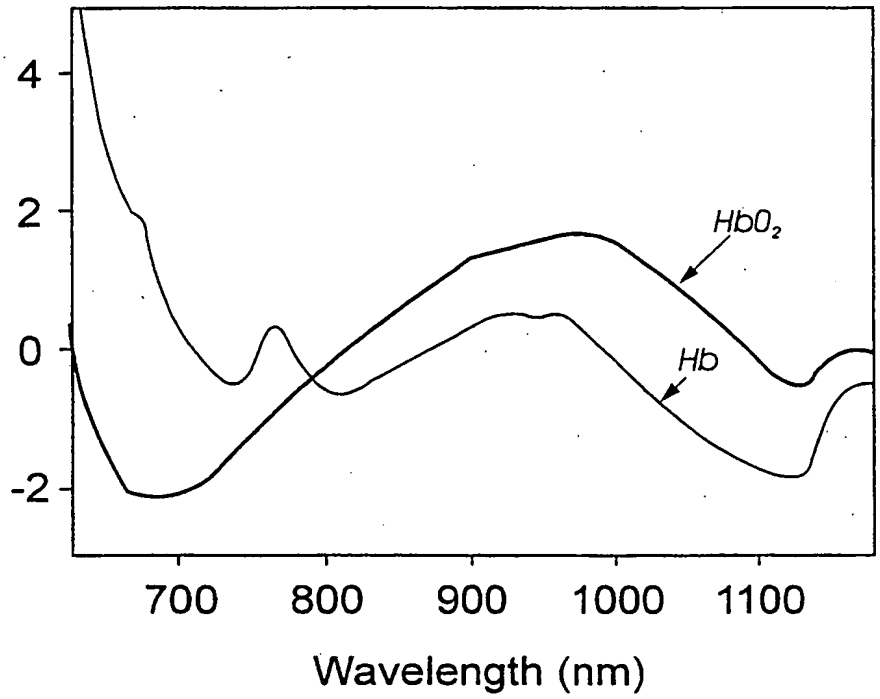

Fig. 7

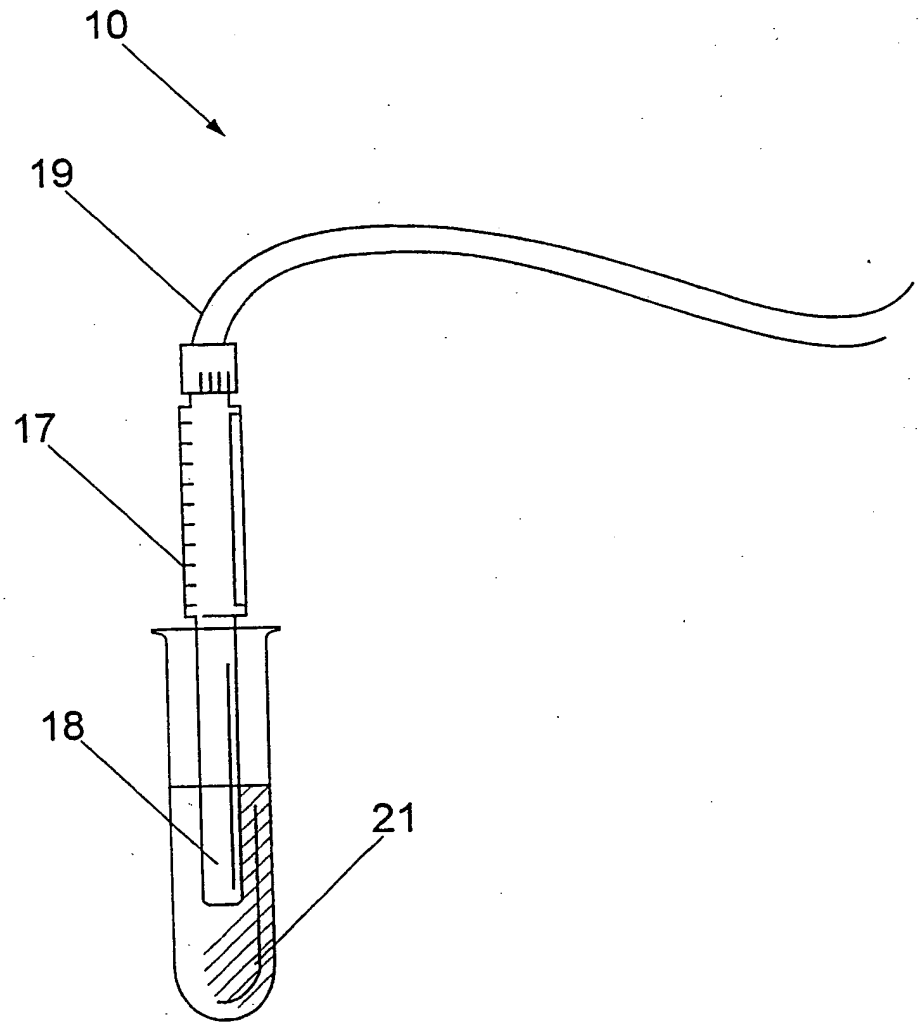

Fig. 8

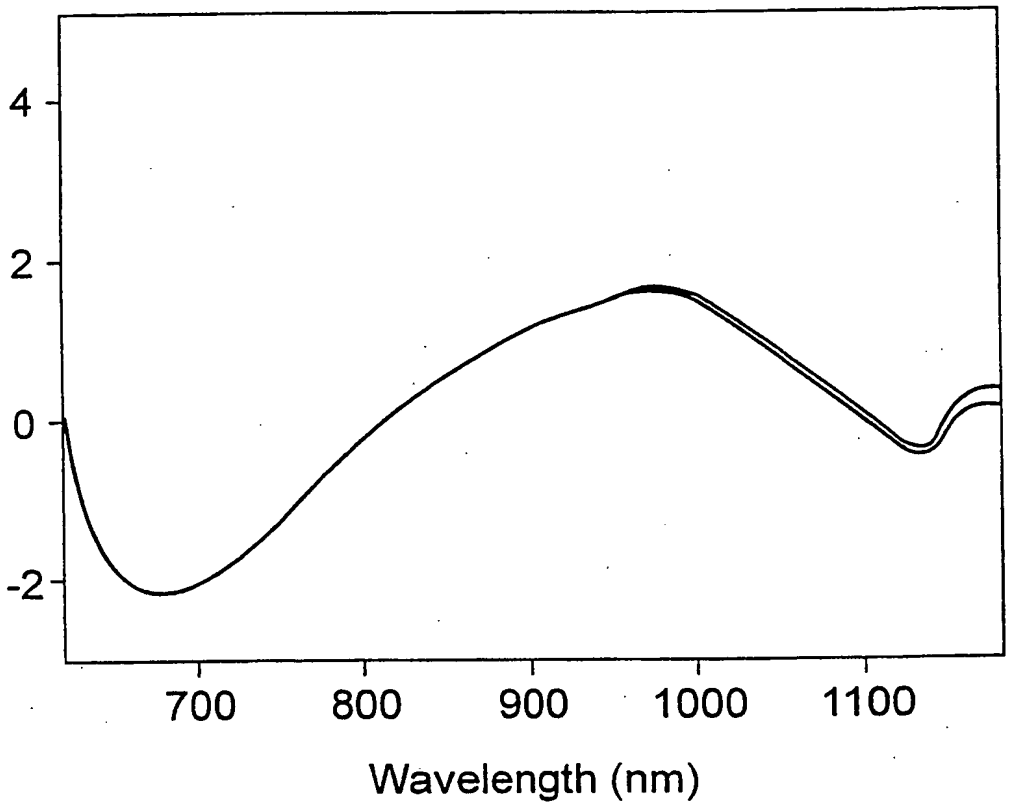

Fig. 9

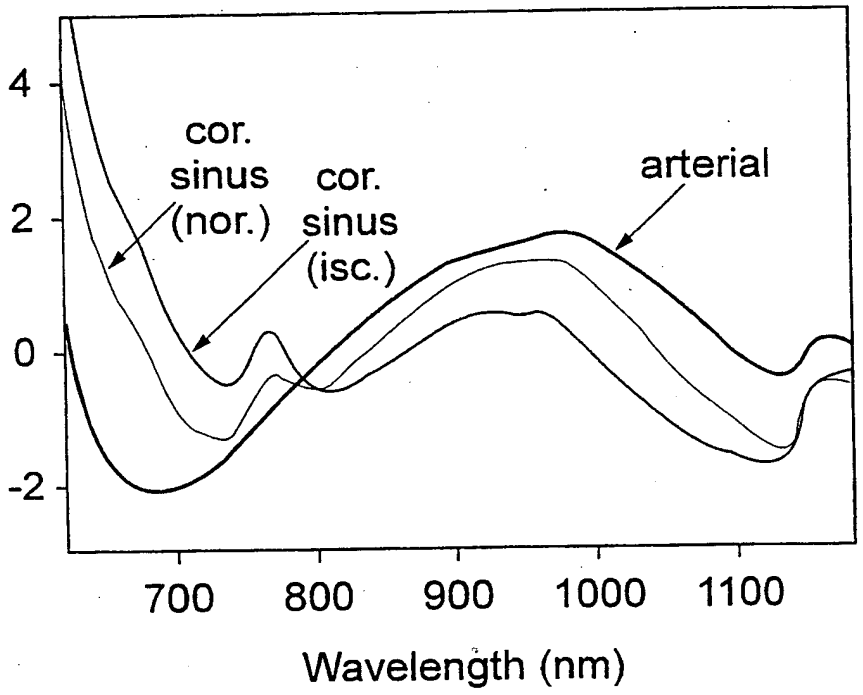

Fig. 10

INTERNATIONAL SEARCH REPORT

Inter 'onal Application No
PC'/GB 99/01811

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61B5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61B

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 590 268 A (MASSACHUSETTS INSTITUTE OF TECHNOLOGY) 6 April 1994 (1994-04-06) the whole document	1-6, 9-11,15, 16,18-28
X	US 5 419 323 A (MASSACHUSETTS INSTITUTE OF TECHNOLOGY) 30 May 1995 (1995-05-30) the whole document	1-5, 9-11, 15-28
A	US 5 683 444 A (HUNTLEY & AL) 4 November 1997 (1997-11-04) column 3, line 1 - line 4 column 7, line 36 - line 52	1,6,7

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 September 1999	30/09/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Lemercier, D

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Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

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Intern· ·nal Application No
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